



## RIBONUCLEASE

### *Molecular Biology*

Worthington Ribonucleases A & B are prepared from bovine pancreas and are offered in several grades to suit various applications. When Ribonuclease A is used to remove RNA during DNA isolation, the enzyme must be free of deoxyribonuclease activity to prevent damage to the DNA. Where other enzymes are used or where the goal is recovery of intact proteins, proteolysis must be prevented. These requirements are satisfied by our Molecular Biology Grade Ribonuclease (Code: RPDF). This and other ribonuclease preparations made by Worthington are described below.

Description	Activity	Code	Cat #	Size	Price
<b>RNase A, <i>Bovine pancreas</i></b> <b>Molecular Biology Grade, DNase and Protease Free.</b> Supplied as a solution containing approximately 5mg/ml in 50% glycerol. Prepared specifically for use in purifying DNA plasmids. Each lot is assayed to be free of DNase and protease. Store at 2 - 8°C. Storage at -20°C is acceptable.	≥ 2,000 units per mg protein	RPDF	LS002131	1 mg	\$ 20.00
			LS002132	5 mg	61.00
			LS002130	Bulk	Inquire
<b>RNase A</b> A highly purified, lyophilized preparation which may contain aggregates as a result of lyophilization but which exhibits same specific activity as RASE (below). Store at 2 - 8°C. <u>Protect from moisture.</u>	≥ 3,000 units per mg dry weight	RAF	LS005649	25 mg	\$ 42.00
			LS005650	100 mg	100.00
			LS005655	Bulk	Inquire
<b>RNase A</b> Monomeric form, purified by method used for RAF (above) and further processed to remove aggregates. Available as a solution in 0.1 M phosphate buffer, pH 7.4 containing 0.1% v/v phenol as a preservative. Store at -20°C.	≥ 3,000 units per mg protein	RASE*	LS005677	25 mg	\$ 40.00
			LS005679	100 mg	96.00
			LS005681	Bulk	Inquire
<b>RNase A</b> Chromatographically purified. Lyophilized. Store at 2 - 8°C. <u>Protect from moisture.</u>	≥ 2,500 units per mg dry weight	R	LS003431	200 mg	\$ 40.00
			LS003433	1 gm	135.00
			LS003435	Bulk	Inquire
<b>RNase A 0.22u Filtered</b> Product Code: R, 0.22u filtered and lyophilized in vials to contain ≥100mg. Store at 2 - 8°C. <u>Protect from moisture.</u>	≥ 2,500 units per mg dry weight	RS	LS003438	1 Vi	\$ 50.00
			LS003440	5 Vi	210.00
<b>RNase T1, <i>Aspergillus oryzae</i></b> Highly purified. Supplied as a solution in 2.8M ammonium sulfate. Store at 2-8°C.	≥ 300,000 units per mg protein	RT1S*	LS01485	100,000 U	\$ 37.00
			LS01487	500,000 U	119.00
			LS01488	Bulk	Inquire
<b>E-RASE™ Ribonuclease A/T1 Blend, DNase and Protease Free.</b> <i>Bovine pancreas/Aspergillus oryzae</i> An optimized blend of 0.5mg/ml RNase A, Code RPDF, and 100,000U/ml RT1, Code RT1S. Suitable for the rapid digestion of RNA in plasmid DNA preps (at a concentration of 10-50 units per typical 5 minute mini-prep treatment), RNA Protection and RT-PCR applications. Supplied as an approximate 10u/μL solution in 50mM acetate, pH 5.0 containing 0.3mM EDTA and 50% glycerol. Store at -20°C.	≥ 8,000 - 12,000 units per ml	RCT*	LS003442	2,500 U	\$ 42.00
			LS003443	10,000 U	125.00
			LS003444	50,000 U	500.00
			LS003446	Bulk	Inquire

\*Requires Special Shipping

(Over) [1.09]

Pancreatic ribonuclease catalyzes cleavage of the phosphodiester bond between the 5'-ribose of a nucleotide and the phosphate group attached to the 3'-ribose of an adjacent pyrimidine nucleotide forming a 2',3'-cyclic phosphate which may then be hydrolyzed to the corresponding 3'-nucleoside phosphate.

Ribonuclease A has a molecular weight of 13,700 daltons. It operates in an optimum pH range of 7.0 - 7.5. Since Molecular Biology Grade Ribonuclease A (Code: RPDF) is essentially free of DNase and protease activities, this product is useful in removing RNA from DNA in nucleic acid work and where other enzymes are used or where intact proteins must be recovered. Ribonuclease is inhibited by heavy metal ions and it is competitively inhibited by DNA. The inhibitory effect of denatured DNA is much greater than that of native nucleic acid. The enzyme is assayed according to the method of Kalnitsky *et al.*, (1959). The rate of hydrolysis of yeast RNA at pH 5.0 is determined by measuring the amount of acid soluble oligonucleotide released under defined conditions.

**Stability:** Molecular Biology Grade product (Code: RPDF) is stable at least 2 years at 2 - 8°C or -20°C. Product Code: RASE is stable 2-3 years at -20°C. Other grades of RNase A are stable 2-3 years at 2 - 8°C.

**Storage:** Codes: R and RAF: Store at 2 - 8° C.  
Protect from moisture.

Code: RASE: Store at -20°C to maintain monomeric form.  
Code: RPDF: Store at 2 - 8°C. Storage at -20°C is acceptable.

**Unit Definitions: RNase A:** 1 unit causes an increase in absorbance of 1.0 at 260 nm at 37°C and pH 5.0 when yeast ribosomal RNA is hydrolyzed to acid soluble oligonucleotides.

**One Kunitz unit equals 50 Worthington units.**

**Code RCT:** One Unit of *E-RASE™* Ribonuclease Blend digests 10ug of yeast RNA in 20ul 10mM Tris-HCl, pH 7.5 containing 15mM NaCl in 30 minutes at 37°C.

**Code: RT1S:** One Unit releases the equivalent of one A260 of acid-soluble products at 37°C, pH 7.5, from yeast RNA in 15 minutes.

#### Technical Notes

Special care should be given to handling of the enzyme because of its affinity for glass surfaces.

The enzyme remains active but aggregates upon lyophilization and in solution at temperatures  $\geq 2^\circ\text{C}$  at low ionic strength.

*Common techniques involving heating solutions of RNase A to inactivate DNase may not be satisfactory since ribonuclease activity may be lost if precipitate formation occurs, and heat inactivated DNase can reactivate over time.* RAF can be used

without treatment in some applications. Although it is not recommended, heat-treating Product Code: RAF in 10mM acetate pH 5.0 with or without 15 mM  $\text{CaCl}_2$  for 15 minutes at 100°C or longer at 80°C will minimize damage to the enzyme. Product may precipitate if heated at neutral pH. Heat treatment of RASE is not possible since the enzyme will precipitate due to the presence of phosphate.

Worthington RPDF grade ribonuclease A is suitable as supplied for applications requiring minimal DNase and protease levels and needs no further treatment.

#### Contaminants

Product Code RPDF is assayed for DNase and protease as follows:

**Deoxyribonuclease** - There is no change in the ethidium bromide electrophoresis band pattern of plasmid DNA following incubation of 20 $\mu\text{g}$  of RPDF for 2 hours at 37°C with 1 $\mu\text{g}$  of plasmid DNA in 10mM Tris, 10mM NaCl and 1mM EDTA at pH 8.0 in a 50 $\mu\text{l}$  volume.

**Protease** - There is no development of digestion zones when 50 $\mu\text{g}$  of RPDF are incubated in a casein agarose plate for 48 hours at 37°C.

#### References

Kalnitsky, G., Hummel, J.P., and Dierks, C.: Some Factors Which Affect the Enzymatic Digestion of Ribonucleic Acid. *J. Biol. Chem.*, 234, 1512 (1959).

### Related Products

**DNase I  
Nucleases, S1 and Micrococcal  
Nucleic Acids  
Proteases**

### On-line Support

**Complete Catalog, Tissue Dissociation  
Guide and Enzyme Manual available  
on-line at:**

**[www.worthington-biochem.com](http://www.worthington-biochem.com)  
[www.tissuedissociation.com](http://www.tissuedissociation.com)**